body, and of the same species as the donor cell, to thereby form a nuclear transfer embryo.

59. (New) A method of cloning a fetus, comprising the steps of:

a. combining a nucleus from a somatic activated donor cell with an activated, enucleated oocyte derived from an oocyte having a first polar body and an extruding second polar body, and of the same species as the donor cell, to thereby form a nuclear transfer embryo;

b. impregnating a mammal of the same species as the nuclear transfer embryo with the nuclear transfer embryo under conditions suitable for gestation of the cloned fetus; and

c. gestating the embryo in step b., thereby causing the embryo to develop into the cloned mammalian fetus.

B10 Review

REMARKS

The remainder of this reply is set forth under appropriate subheadings for the convenience of the Examiner.

Applicants' Invention

Applicants' claimed invention is directed to a method of producing a nuclear transfer embryo, including combining a genome from a somatic activated donor cell with an activated, enucleated oocyte (as evidenced by a first polar body and an at least partially extruding second polar body, such as an oocyte in telophase II) of the same species as the activated donor cell.

In one embodiment, the method is directed to cloning an animal by use of the nuclear transfer embryo. The genome can be genetically engineered prior to formation of the transgenic nuclear transfer embryo. In another embodiment, the method includes cloning an animal wherein the genome of the donor nucleus encodes the protein of interest. The protein can be, for example, a heterologous protein.

In another embodiment, the method of the invention includes enucleating an oocyte having a meiotic spindle apparatus, including exposing the oocyte to at least one compound that destabilizes the meiotic spindle apparatus. The enucleated oocyte then can be employed to form a nuclear transfer embryo.

Advantages of Applicants' Invention

Applicants have discovered that, surprisingly, developmentally competent nuclear transfer embryos can be formed by combining somatic activated donor cells with activated, enucleated oocytes, as evidenced by an at least partially extruding second polar body, such as an oocyte in telophase II. Further, use of such cytoplasts may have several practical and biological advantages. For example, use of activated cytoplasts facilitates efficient enucleation, because, for example, they include a protrusion of the plasma membrane by the second polar body. The protrusion generally avoids the necessity for staining and ultraviolet illumination to localize nuclear chromatin. Moreover, enucleation of activated oocytes enables removal of minimal cytoplasmic material and selection of a synchronous group of activated donor cytoplasts. Applicants' method of forming a nuclear transfer embryo also allows for preparation of a highly homogeneous group of donor nuclei to be synchronized with the cell cycle of the cytoplast. Further, when used for embryo reconstruction, these populations showed a higher rate of embryonic development *in vitro*. Thus, reconstructed embryos comprised of a synchronously activated cytoplast and karyoplast (i.e. donor cell) were developmentally competent.

In addition, nuclear transfer of somatic cells allows for selection of the appropriate transgenic cell line before generation of cloned transgenic embryos. This is particularly important in the cases where several proteins are to be coexpressed by the transgenic mammary gland. For example, the availability of several completely identical transgenic females producing recombinant human ATIII could help determine the extent of variation in the carbohydrate structure of this protein, as it is produced by the mammary gland. Thus, it may be feasible to improve characteristics of the recombinant proteins produced in the transgenic animal system by varying environmental factors (e.g. nutrition) or to increase the milk volume yield of lactation-

induction protocols to diminish further the time necessary to obtain adequate amounts of recombinant proteins for preclinical or clinical programs.

Applicants' discovery that somatic donor nuclei can be combined with activated enucleated oocytes to form a nuclear transfer embryo enables the benefits of somatic cells and such oocytes to be realized in a single cloning protocol. Therefore, advantages are provided over known methods of cloning that employ either somatic (e.g. differentiated) donor cells in combination with enucleated oocytes in metaphase II, or known methods that employ activated enucleated oocytes with donor cells that are not somatic cells (e.g. blastomeres).

Amendments to the Specification

The specification has been amended at page 1 to acknowledge Government support. Also, a paragraph of the specification has been amended at page 11, line 2, to eliminate reference to "cytochalasins." Inclusion of cytochalasins as an agent that affects differential segregation of polar bodies in an oocyte was an error. Support for amendment of the specification to correct this error can be found at: page 4, lines 9-12; page 10, lines 14-16; page 22, lines 7 and 8; and original Claims 33 and 37. This paragraph was also amended to specify nuclear chromatin, instead of chromatin to further clarify the invention. Support for "nuclear chromatin" can be found, for example, in the specification on page 23, lines 6-10. No new matter has been added.

Claim Amendments

Independent Claims 1, 8, 11, 21 and 25 have been amended to specify that the activated donor cell is a somatic cell, and that the activated, enucleated oocyte is in telophase II (i.e., evidenced by a first polar body and an extruding second polar body). Support for these amendments can be found in canceled Claims 3, 6, 10, 18 and 27. Further support can be found in the specification at, for example, page 7, lines 5-8 and page 8, lines 7-9.

Independent Claims 1, 8, 11, 21 and 25 also have been amended to state that the somatic activated donor cell and that the activated, enucleated oocyte in telophase II are from mammals, and of the same species, and that the mammal in which the nuclear transfer embryo is implanted

also is of the same species. Support can be found in the specification at page 5, lines 11-16, and in the exemplification portion of the specification, starting at page 22, line 18.

The term "genome" in claims 1, 8, 11, 19-21, and 29 has been substituted with the word "nucleus." Support for this limitation can be found in the specification at page 11, lines 26 and 27.

New claims 39-43 parallel existing claims 1, 2, 4, 5 and 7, as amended, but specify that the mammal is a mammalian fetus. New Claims 47 and 51 also specify a mammalian fetus. New Claim 55 and 59 also specify that the method is directed to cloning a fetus. Support for the additional element of a fetus can be found in the specification at page 12, line 11.

New claims 44 and 45 parallel existing Claim 1, but specify that the mammal is a non-human mammal and a transgenic non-human mammal, respectively. New Claims 48 and 49 specify a mammal and a transgenic mammal, respectively. Support for these limitations can be found in the specification at page 5, lines 11-15, and at page 12, lines 24-26.

New claims 52-54, 56 and 57 are directed to producing or cloning an animal or a transgenic animal. Support for this element can be found in original Claims 1 and 8.

New Claim 48-51 and 56-59 state that the activated, enucleated oocyte is derived from an oocyte that has a first polar body and an extruding second polar body. Support for use of the term "activated" can be found in the specification at page 8, lines 7-10, which state that the oocytes used in the present invention are activated oocytes, as opposed to oocytes in metaphase II, which are considered to be in a resting state. Support for the limitation that the oocyte to be enucleated has a first polar body and an extruding second polar body can be found in the specification at page 10, lines 14-16 and in Example 2, at page 30, lines 8-15 which states that oocytes were employed under an "activated telophase II protocol." Oocytes were selected for the "activated telophase II protocol" if the oocyte had one polar body and evidence of a second polar body, as compared to oocytes in metaphase II, which had only one evident polar body. As stated at page 30, lines 9-15:

> Cumulus-free oocytes were divided into two groups: oocytes with only one polar body evident (metaphase II stage) and the activated telophase II protocol (oocytes with one polar body and evidence of an extruding second polar body). Oocytes in

> telophase II were cultured in M199 + 10% FBS for 3 to 4 hours. Oocytes that had activated during this period, as evidenced by a first polar body and a partially extruded second polar body, were grouped as culture induced, calcium activated telophase II oocytes (Telophase II-Ca^{+2}) and enucleated.

Enucleated "Telophase II-Ca^{+2}" oocytes were employed for reconstructed embryos that were subsequently implanted and successfully brought to term, as identified in Table 2, at page 38 of the specification. Oocytes selected for the "activated telophase II protocol" would be considered to be in telophase II or, at least , in anaphase, which is defined in Stedman's Medical Dictionary, 26th edition, on page 71, for example, as :

> In the second division of meiosis the centromere divides, and the two chromatids separate with one moving to each pole.

As stated above, oocytes exhibiting evidence of an extending second polar body would not be considered to be in metaphase II since such oocytes considered to be in metaphase II have only a single polar body.

Other, minor claim amendments have been made to correct grammatical errors or make necessary changes which are self-evident.

Denial of Benefit of Priority for Claims 31-38 to U.S.S.N. 60/106,728, filed November 2, 1998

Applicants acknowledge that the subject matter of Claims 31-38 is limited to a priority date of August 17, 1999, which is the filing date of United States Serial Number 60/149,317, filed August 17, 1999. The subject matter of Claims 31-38 is directed to enucleating an oocyte having a meiotic spindle apparatus, including the step of exposing the oocyte to at least one compound that destabilizes the meiotic spindle apparatus. The subject matter of these claims is not disclosed in U.S.S.N. 60/106,728, filed November 2, 1998.

Rejection of Claims under 35 U.S.C. § 112, First Paragraph

Claims 1-37 stand rejected under 35 U.S.C. § 112, first paragraph, as not being supported by any specification that would enable any person skilled in the art to which it pertains, or with

which is most nearly connected, to practice the invention commensurate in scope with these claims. Specifically, the Examiner stated that designation that the oocyte must be in telophase II is critical to the invention and that there is no evidence of record, or in the art, that oocytes in other activated stages, such as telophase I, would provide the correct environment to reprogram the donor nucleus. Similarly, the Examiner noted that, in the specific examples of the application, the oocyte cytoplast, donor nucleus and recipient female are all of the same species. The Examiner stated that the specification, through the discussion and the working examples, provides guidance for this scenario.

In response to the Examiner's rejection, independent Claims 1, 8, 11, 21 and 25 have been amended to specify that the activated, enucleated oocyte is in telophase II and that the oocyte cytoplast, donor cell and recipient animal are all of the same species. Independent Claim 31 is directed to a method of enucleating an oocyte and makes no reference to forming a nuclear transfer embryo or of implanting the nuclear transfer embryo in an animal. Therefore, independent Claim 31 and claims dependent from independent Claim 31 do not need to be amended.

New claims 48-51 and 56-59 identify the oocyte being derived from an activated oocyte as having a first polar body and an extruding second polar body. As set forth in the specification at page 30, lines 8-20, oocytes selected for use under the "activated telophase II protocol", had one polar body and evidence of an extruding second polar body, as opposed to oocytes in metaphase II, which had only one evident polar body. Enucleated oocytes derived from oocytes have evidence of an extruding second polar body were designated as "Telophase II-Ca^{+2}" and successfully employed to form reconstituted embryos that were brought to term. (See Table 2 at page 38). Therefore, Applicants have demonstrated, and provided reasonable enablement for, methods that employ enucleated oocytes derived from oocytes that have one polar body as evidence of an extruding second polar body, as claimed.

As amended, pending Claims 1-37 meet the requirements of 35 U.S.C. § 112, first paragraph.

Rejection of Claims 1-5, 7-9, 11, 14, 15, 19 and 20 under 35 U.S.C. § 102(b)

Claims 1-5, 7-9, 11, 14, 15, 19 and 20 stand rejected under 35 U.S.C. § 102(b) as being clearly anticipated by international publication number WO 97/07668, published March 6, 1997 (hereinafter Campbell '668). In particular, the Examiner stated that Campbell '668 teaches production of a nuclear transfer embryo by nuclear transfer of a G_1 somatic cell nucleus by fusion of the nucleus with an activated enucleated oocyte after the disappearance of maturation (or meiosis) promoting factor (MPF) activity and that the oocyte can be in metaphase II prior to activation. The Examiner cites page 4, lines 5-7 of Campbell '668 for support. The Examiner also stated that Campbell '668 teaches transfer of the reconstructed embryo to a surrogate mother, that the donor nucleus may be genetically modified, that the donor cell can be a fetal fibroblast, and that the donor nucleus can be introduced into the oocyte by microinjection. The Examiner also stated that the oocyte can be enucleated by aspiration or by x-irradiation.

In contrast to the Examiner's statement, Campbell '668 does not teach nuclear transfer of a G_1 somatic cell nucleus by fusion of the nucleus with an activated enucleated oocyte after the disappearance of MPF activity. The complete sentence, of which the Examiner cited the portion at page 4, lines 5-7, reads as follows:

> In reconstructed embryos correct ploidy can be maintained in one of two ways; firstly by transferring nuclei at a defined cell cycle stage, e.g. diploid nuclei of cells in G1, into metaphase II oocytes at the time of activation; or secondly by activating the recipient oocyte and transferring the donor nucleus after the disappearance of MPF activity. (Emphasis added).

This sentence does not teach a single method wherein donor nuclei from cells in G_1, regardless of whether the cells are somatic cells, to recipient oocytes following disappearance of MPF activity. Rather, two distinct methods are taught by this sentence. Further, the following sentence, at page 4, lines 7-12, makes clear that the second method, wherein the recipient oocyte is an activated oocyte and wherein there is no MPF activity, is not combined with a somatic cell, but rather with a blastomere:

> In sheep this latter approach has yielded an increase in the frequency of development to the blastocyst stage from 21% to 55% of reconstructed embryos when using blastomeres from 16 cell embryos as nuclear donors (Campbell, *et al., Biol. Reprod. 50*:1385-1393 (1994)).

Blastomeres are not somatic cells; they are undifferentiated pluripotent cells, some of whose lineages give rise to germ cells. Further, there is no disclosure or suggestion in Campbell, *et al., Biol. Reprod, 50*:1385-1393 (1994) (attached as Exhibit A) of combining an activated, enucleated oocyte with low MPF activity, with the nucleus of an activated somatic, or differentiated, donor cell.

Campbell '668 teaches an improvement in the frequency of development of reconstructed embryos by addressing reprogramming activity by the oocyte consequent to high levels of MPF. These activities are summarized at page 3, lines 18-30:

> Immediately upon fusion the donor nuclear envelope breaks down (NEBD), and the chromosomes prematurely condense (PCC). These effects are catalyzed by a cytoplasmic activity termed maturation/mitosis/meiosis promoting factor (MPF). This activity is found in all mitotic and meiotic cells reaching a maximal activity at metaphase. Matured mammalian oocytes are arrested at metaphase of the 2nd meiotic division (metaphase II) and have high MPF activity. Upon fertilization or activation MPF activity declines, the second meiotic division is completed and the second polar body extruded, the chromatin then decondenses and pronuclear formation occurs.

The invention described by Campbell '668 exploits the reprogramming portion of metaphase II oocytes. Specifically, Campbell '668 teaches, as stated at page 4, lines 14-26, with reference to the teachings of Campbell '668 identified by the Examiner:

> These improvements in the frequency of development of reconstructed embryos have as yet not addressed the question of nuclear reprogramming. During development certain genes become "imprinted" i.e. are altered such that they are no longer transcribed. Studies on imprinting have shown that this "imprinting" is removed during germ cell formation (i.e.

> reprogramming). One possibility is that this reprogramming is affected by exposure of the chromatin to cytoplasmic factors which are present in cells undergoing meiosis. This raises the question of how we may mimic this situation during the reconstruction of embryos by nuclear transfer in order to reprogram the developmental clock of the donor nucleus.

Campbell '668 then goes on to teach away from employing oocytes where MPF activity has disappeared to form reconstructed embryos. Specifically, Campbell '668 states at page 4, lines 28-33:

> It has now been found that nuclear transfer into an oocyte arrested in metaphase II can give rise to a viable embryo if normal ploidy (i.e. diploidy) is maintained and if the embryo is not activated at the time of nuclear transfer. The delay in activation allows the nucleus to remain exposed to the recipient cytoplasm.

Campbell '668 specifically states that the oocyte should not be activated at the time of nuclear transfer. For example, as stated at page 10, lines 18-22:

> After enucleation, the donor nucleus is introduced either by fusion to donor cells under conditions which do not induce oocyte activation or by injection under non-activating conditions.

Also, as stated at page 10, lines 29-30:

> Activation should not take place at the time of fusion.

Campbell '668 also teaches that the MPF activity of the metaphase II oocytes should be high. For example, as stated at page 12, lines 10-20:

> In practice, it is best to enucleate and conduct the transfer as soon as possible after the oocyte reaches metaphase II . . . . High MPF activity is desirable.

Campbell '668 teaches that viability of the embryo is contingent upon exposing the donor nucleus to recipient cytoplasm without activation of the cytoplasm for a period of time. As stated at page 12, lines 21-28:

> Subsequently, the fused reconstructed embryo, which is generally returned to the maturation medium, is maintained without being activated so that the donor nucleus is exposed to the recipient cytoplasm for a period of time sufficient to allow the reconstructed embryo to become capable, eventually, of giving rise to a live birth (preferably of a fertile offspring).

Applicants have amended independent Claims 1, 8 and 11 to include the limitations that the activated donor cell is a somatic activated donor cell, and that the activated, enucleated oocyte is in telophase II. Support for these amendments to the rejected independent claims is recited above.

There is no disclosure in Campbell '668 of a method of cloning an animal or a method of forming a nuclear transfer embryo that includes combining a somatic activated donor cell with an activated, enucleated oocyte in telophase II, as is claimed by Applicants. Therefore, Applicants' rejected independent claims and the claims that depend from them are not anticipated by Campbell '668. These claims meet the requirements of 35 U.S.C. § 102(b) in view of Campbell '668.

Rejection of Claims 31, 32 and 34 under 35 U.S.C. § 102(b)

Claims 31, 32 and 34 stand rejected under 35 U.S.C. § 102(b) as being clearly anticipated by Bordignon, *et al., Molecular Reproduction and Development, 49*:29-36 (1998). In particular, the Examiner stated that Bordignon, *et al.* teach enucleation of telophase nuclei by incubating the nuclei with cytochalasin B. The Examiner stated that both the meiotic spindle apparatus and chromosomes were destabilized, as evidenced by a fragment of chromatin in the first polar body of the oocyte.

Applicants' claimed invention, as set forth in independent Claim 31, is directed to a method of enucleating an oocyte having a meiotic spindle apparatus, wherein the oocyte is exposed to at least one compound that destabilizes the meiotic spindle apparatus.

Exhibit B is an article, Krishan, A., *J. Cell Biol., 54*:657-664 (1972), that describes the action of cytochalasin B on cells. Specifically, as stated at page 663 of Krishan:

> Our time-lapse observations, which essentially show that in tissue culture cells cyto-B [Cytochalasin-B] does not inhibit cleavage furrow formation and progression but rather the moving away of the daughter cells, are more compatible with studies showing that cyto-B affects the cell membrane than with studies suggesting a cyto-B-induced disruption and disorganization of the contractile microfilaments of the cell.

Therefore, as described in Krishan, cytochalasin B does not affect the formation of contractile microtubules, the cytoskeleton components of the meiotic/mitotic spindle. Rather, Krishan suggests that cytochalasin B's primary effect is to soften the cell membrane, and not to disorganize microfilaments. Microfilaments are not a component of the meiotic/mitotic spindle, which is the focus of the invention.

Cytochalasin B often is employed, as it was in the protocol employed in Bordignon, *et al.*, to soften the cell membrane of oocytes. In contrast to the statement made by the Examiner, Fig. 1 of Bordignon, *et al.* does not shown chromatin fragmentation consequent to exposure of an oocyte to cytochalasin B. Rather, Bordignon, *et al.* describe two oocyte enucleation protocols. The first, or "standard protocol," is set forth at page 31, in the first column of the page, starting at the second sentence under the heading "Oocyte Reconstruction Protocols." The second, the "telophase" group, is set forth in the second column of page 31 and continues on to page 32. Both protocols employ cytochalasin B. Figure 1 shows photographs taken of the second protocol, using only oocytes in the "telophase" group. Specifically, as stated in the second column of page 34 of Bordignon, *et al.*:

> In our study, approximately a third of the total cytoplasmic volume was removed to achieve a 60 % enucleation of metaphase oocytes. On the other hand, enucleation at the telophase stage relies on a

> precise positioning of the chromatin as it is being expelled in the second polar body, enabling successful enucleation of practically all manipulated oocytes with minimal removal of cytophase.

There is no disclosure or suggestion that cytochalasin B had any effect on the meiotic spindle apparatus of either the arrested-metaphase or activated telophase oocyte.

Pretreatment of an oocyte by exposure to cytochalasin B does not anticipate Applicants' claimed method, as set forth in rejected Claims 31, 32 and 34. Therefore, these claims meet the requirements of 35 U.S.C. § 102(b) in view of Bordignon, *et al.*

Rejection of Claims 35, 36 and 38 under 35 U.S.C. § 102(b)

Claims 35, 36 and 38 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Bordignon, *et al.* The Examiner stated that Bordignon, *et al.* teach enucleation of telophase nuclei which have a meiotic spindle apparatus, by incubating the nuclei in cytochalasin B. The Examiner also stated that the oocytes were activated by exposure to ethanol.

As discussed above, cytochalasin B does not operate as an agent that destabilizes the meiotic spindle apparatus, or more specifically, microtubules of an oocyte. Therefore, Bordignon, *et al.* also do not anticipate the subject matter of rejected Claims 35, 36 and 38. Rejected Claims 35, 36 and 38 meet the requirements of 35 U.S.C. § 102(b) in view of Bordignon, *et al.*

Rejection of Claims 1, 6, 8, 10, 11 and 18 under 35 U.S.C. § 103(a)

Claims 1, 6, 8, 10, 11 and 18 stand rejected under 35 U.S.C. § 103(a) as being unpatentable in view of Campbell '668 in view of Wu, *et al., Biol. of Reprod., 56*:253-259 (1997) (hereinafter "Wu, *et al.*"). In particular, the Examiner stated that Campbell '668 teaches production of cloned non-human mammals by the nuclear transfer of a G_1 somatic cell nucleus by fusion of the nucleus with an activated enucleated oocyte after the disappearance of MPF activity, followed by transfer of the reconstructed embryo to a surrogate mother. The Examiner also stated that Wu, *et al.* supplement Campbell '668 by stating that at the anaphase/telophase stage of meiosis, oocytes have an abrupt reduction of MPF activity. Therefore, according to the

Examiner, in would have been obvious to the ordinary artisan to modify the method of Campbell '668 by using a telophase II oocyte, which has a low level of MPF, as taught by Wu, *et al.*, given the motivation of Campbell that a suitable recipient oocyte is one that is preactivated having low levels of MPF.

As discussed above, the passage recited by the Examiner from Campbell '668 does not teach formation of a reconstructed embryo that is the combination of a G_1 somatic cell nucleus and an activated enucleated oocyte after the disappearance of MPF activity. Rather, the passage refers to two distinct methods, one of which is the combination of a G_1 donor nucleus into a metaphase II oocyte and the other being combination of a donor cell from a blastomere with an enucleated oocyte following disappearance of MPF activity.

The method that is the subject of the Campbell '668 publication is described as an improvement over the two methods set forth in the passage recited by the Examiner. Specifically, Campbell '668 teaches a method of forming a reconstructed embryo that is the combination of a differentiated donor nucleus and an oocyte in metaphase II that has a high level of MPF activity. Campbell '668 specifically teaches away from employing an oocyte in any stage other than metaphase II and, in particular, teaches away from employing an oocyte having a low level of MPF activity. For example, as stated at page 12, lines 10-12 and at lines 19-20:

> In practice, it is best to enucleate and conduct the transfer as soon as possible after the oocyte reaches metaphase II . . . . High MPF activity is desirable.

Further, Campbell '668 also teach that donor nuclei preferably are in the G_0 phase of the cell cycle. (See page 7, lines 20-24).

Wu, *et al.* teach variation in the level of maturation promoting factor in oocytes during maturation. As indicated by the Examiner, Wu, *et al.* teach that there is an abrupt reduction in MPF activity during presumptive anaphase and telophase stages.

Wu, *et al.* do not remedy the deficiencies of Campbell '668. In particular, as with Campbell '668, there is no disclosure or suggestion in Wu, *et al.* of employing an enucleated oocyte in telophase II as a recipient for a somatic activated donor cell to form a nuclear transfer

embryo, as is claimed by Applicants. Therefore, Applicants' claimed method of cloning an animal, and method for forming a nuclear transfer embryo, is not obvious in view of Campbell '668 or Wu, *et al.*, taken either separately or in combination.

The subject matter of independent Claims 1, 8 and 11 and of dependent Claims 6, 10 and 18 meets the requirements of 35 U.S.C. § 103(a) in view of Campbell '668 and Wu, *et al.*, taken either separately or in combination.

Rejection of Claims 11-13 under 35 U.S.C. § 103(a)

Claims 11-13 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Campbell '668 in view of U.S. 5,945,577, issued on August 31, 1999 to Stice, *et al.* (hereinafter "Stice, *et al.*"). In particular, the Examiner stated that Campbell '668 teaches production of a nuclear transfer embryo by nuclear transfer of a G_1 somatic cell nucleus by fusion of the nucleus with an activated enucleated oocyte after the disappearance of MPF activity. The Examiner further stated that Stice, *et al.* supplement Campbell '668 in teaching oocyte activation by elevating the number of calcium ions and by reduction of phosphorylation. Therefore, according to the Examiner, it would have been obvious to the ordinary artisan to modify the method of Campbell by activating the oocyte using an increased concentration of calcium and by incubation with a calcium inophore and incubation in the presence of DMAP which is a phosphorylation inhibitor, as taught by Stice, *et al.*

As discussed above, and in contrast to the Examiner's statement, Campbell '668 does not teach production of a nuclear transfer embryo by nuclear transfer of a G_1 somatic cell nucleus by fusion of the nucleus with an activated enucleated oocyte after the disappearance of MPF activity.

Stice, *et al.* teach nuclear transfer of differentiated cell nuclei into enucleated oocytes that are in metaphase II. Stice, *et al.* do not remedy the deficiencies of Campbell '668. Specifically, there is no disclosure or suggestion in Stice, *et al.* of employing enucleated oocytes in telophase II as recipients for somatic activated donor cell nuclei, as in Applicants' claimed method. Therefore, regardless of the use of calcium ions or decreased phosphorylation in the oocyte,

neither Campbell '668 nor Stice, *et al.*, taken either separately or in combination, disclose or suggest Applicants' claimed method, as set forth in Claims 11-13.

Claims 11-13 meet the requirements of 35 U.S.C. § 103(a) in view of Campbell '668 and Stice, *et al.*, taken either separately or in combination.

Rejection of Claims 21-30 under 35 U.S.C. § 103(a)

Claims 21-30 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Campbell '668 in view of Wu, *et al.* and Ebert, *et al.*, *Bio/Technology* 9:835-838 (1991). In particular, the Examiner stated that Campbell '668 teaches production of a nuclear transfer embryo by nuclear transfer of a G_1 somatic cell nucleus by fusion of the nucleus with an activated enucleated oocyte after the disappearance of MPF activity, followed by transfer of the reconstruction embryo to a surrogate mother to form clones of non-human animals. The Examiner also stated that Ebert, *et al.* supplements Campbell '668 in teaching that the production of human tPA in the milk goats, where the goat's genome comprises a DNA sequence encoding human tPA that is operably linked to a mouse WAP promoter. Further, the Examiner stated that Wu, *et al.* supplements the teaching of Campbell '668 by stating that, at the anaphase/telophase stage of meiosis, oocytes have an abrupt reduction in MPF activity. Therefore, according to the Examiner, at the time of the instant invention it would have been obvious to the ordinary artisan to modify the method of Campbell '668 by using a telophase II oocyte that has low levels of MPF, as taught by Wu, *et al.*, given the motivation of Campbell '668 that the recipient oocyte needs to have low levels of MPF, and by using as a nuclear donor the cell of a transgenic goat, as taught by Ebert, *et al.*

As discussed above, there is no disclosure in Campbell '668 of combining a G_1 somatic donor cell nucleus with an enucleated oocyte exhibiting low levels of MPF activity, regardless of the stage of meiosis. On the contrary, Campbell '668 teach away from employing enucleated oocytes having low or no level of MPF activity. Campbell '668 also teaches away from using an activated donor nucleus. Further, neither Wu, *et al.* nor Ebert, *et al.*, either separately or in combination, remedy these deficiencies of Campbell '668; none of the references cited by the Examiner, taken either separately or in combination, discloses or suggests combining a somatic

activated donor nucleus with an enucleated oocyte in telophase II, as claimed by Applicants. Therefore, Applicants' claimed method, as set forth in rejected Claims 21-30, meets the requirements of 35 U.S.C. § 103(a) in view of these references, taken either separately or in combination.

Rejection of Claims 31, 33, 35 and 37 under 35 U.S.C. § 103(a)

Claims 31, 33, 35 and 37 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Bordignon, *et al.* In particular, the Examiner stated that Bordignon, *et al.* teach enucleation of telophase nuclei, which have a meiotic spindle apparatus, by incubating nuclei in cytochalasin B. The Examiner further stated that, at the time of the instant invention, it would have been obvious to the ordinary artisan to substitute any tubulin destabilizing agent for cytochalasin B, which functions to destabilize chromosomes and centrioles by destabilizing microfilaments. The Examiner also stated that motivation is provided by Bordignon, *et al.* stating that their method provides for enucleation without the use of DNA stains or UV irradiation with a result of great blastocyst achievement.

As discussed above, cytochalasin B does not destabilize the meiotic spindle apparatus of an oocyte. Rather, cytochalasin B is employed to soften the cell membrane of an oocyte, thereby facilitating mechanical enucleation by use of a micropipet.

There is no disclosure or suggestion in Bordignon, *et al.* of employing a compound that destabilizes the meiotic spindle apparatus of an oocyte as part of a method of enucleating the oocyte, as is claimed by Applicants. Therefore, Applicants' method, as claimed in Claims 31, 33, 35 and 37, meets the requirements of 35 U.S.C. § 103(a) in view of Bordignon, *et al.*

Rejection of Claims 11, 16 and 17 under 35 U.S.C. § 103(a)

Claims 11, 16 and 17 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Campbell '668. Specifically, the Examiner stated that Campbell '668 teaches production of cloned non-human mammals by nuclear transfer of a G_1 somatic cell nucleus by fusion of the nucleus with an activated enucleated oocyte after disappearance of MPF activity, followed by transfer of the reconstructed embryo to a surrogate mother. The Examiner stated that Campbell

'668 does not teach a method of culture by first serum starvation of the cells, followed by growth at normal serum conditions, but that it would have been obvious to the ordinary artisan to modify the teachings of Campbell '668 to do so.

As discussed above, Campbell '668 does not disclose or suggest formation of a nuclear transfer embryo by combining a genome from a somatic activated donor cell with an activated, enucleated oocyte in telophase II. Therefore, the subject matter of independent Claim 11 meets the requirements of 35 U.S.C. § 103(a) in view of Campbell '668. Claims 16 and 17 both depend, directly or indirectly, from independent Claim 11. Therefore, these claims also meet the requirements of 35 U.S.C. § 103(a) in view of Campbell '668.

SUMMARY AND CONCLUSIONS

Applicants have amended Claims 1, 8, 11, 21 and 25 to include the limitations that the activated donor cell is a somatic activated donor cell, that the activated, enucleated oocyte is in telophase II (i.e., evidenced by a first polar body and an extruding second polar body), and that the donor cell, the enucleated oocyte and the animal in which the resulting nuclear transfer embryo is implanted are all of the same species. As amended, all of the claims meet the requirements of 35 U.S.C. § 112, first paragraph, 102(b) and 103(a) in view of the references cited by the Examiner, taken either separately or in combination.

If the Examiner feels that a telephone conference would expedite prosecution of this application, she is respectfully invited to call the undersigned Attorney.

Respectfully submitted,

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MARKED UP VERSION OF AMENDMENTS

Specification Amendments Under 37 C.F.R. § 1.121(b)(1)(iii)

Replace the paragraph at page 10, line 19 through page 11, line 13 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

The oocyte can be rendered functionally inactive also by chemical methods. Methods of chemically inactivating the DNA are known to those of skill in the art. For example, chemical inactivation can be preformed using the etopsoide-cycloheximide method as described in Fulka and Moore, *Molecul. Reprod. Dev., 34*:427-430 (1993). The present invention includes enucleating the genome of an oocyte by treating the oocyte with a compound that will induce the oocyte genome (e.g., nuclear chromatin) to segregate into the polar bodies during meiotic maturaton thereby leaving the oocyte devoid of a functional genome, and resulting in the formation of a recipient cytoplast for use in nuclear transfer procedures. Examples of agents that will effect such differential segregation include agents that will disrupt 1) cytoskeletal structures including, but not limited to, Taxol® (e.g., paclitaxel), demecolcine, phalloidin, colchicine, nocodozole, [cytochalasins,] and 2) metabolism including, but not limited to, cycloheximide and tunicamycin. In addition, exposure of oocytes to other agents or conditions (e.g. increased or decreased temperature, pH, osmolality) that preferentially induce the skewed segregation of the oocyte genome so as to be extruded from the confines of the oocyte (e.g., in polar bodies) also are included in the preferred method. See, for example, methods to include changes in the cytoskeleton and metabolism of cells, methods that are known to those in the art Andreau, [JM<] J.M. and Timasheff, [SN, Proc. Nat. Acad. Sci.] S.N., *Proc. Nat. Acad. Sci.* 79:6753 (1982), Obrig, [TG et al, J. Biol. Chem.] T.G., *et al. J. Biol. Chem.* 246:174 (1971), Duskin, D. and Mahoney, [WC, J. Biol. Chem.] W.C., *J. Biol. Chem.* 257:3105 (1982), Scialli, [AR et al, Teratogen, Carcinogen, Mutagen] A.R., *et al. Teratogen, Carcinogen, Mutagen* 14:23 (1994), Nishiyama, I and Fujii, [T, Exp. Cell Res.] T., *Exp. Cell Res.* 198:214 (1992), Small, [JV et al, J. Cell Sci.] J.V., *et al., J. Cell Sci.* 89:21 (1988), Lee, [JC et al, Biochem.] J.C., *et al, Biochem.* 19:6209 (1980).

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

1. (Amended) A method of cloning a mammal [an animal], comprising the steps of:

a. combining a [genome] nucleus from [an] a somatic activated donor cell with an activated, enucleated oocyte in telophase II and of the same species as the donor cell, to thereby form a nuclear transfer embryo; [and]

b. impregnating a mammal [an animal] of the same species as the nuclear transfer embryo with the nuclear transfer embryo [in] under conditions suitable for gestation of the cloned [animal] mammal; and

c. gestating the embryo in step b., thereby causing the embryo to develop into the cloned mammal.

4. (Amended) The method of Claim [3] 2, wherein the somatic cell is an adult somatic cell or an embryonic somatic cell.

5. (Amended) The method of Claim [3] 2, wherein the somatic cell is a fibroblast cell or an epithelial cell.

8. (Amended) A method of producing a transgenic mammal [animal], comprising the steps of:

a. combining a genetically engineered [genome] nucleus from [an] a somatic activated donor cell with an activated, enucleated oocyte in telophase II and of the same species as the donor cell, to thereby form a transgenic nuclear transfer embryo; [and]

b. impregnating [an animal] a mammal of the same species as the nuclear transfer embryo with the transgenic nuclear transfer embryo [in] under conditions suitable for gestation of the transgenic [animal] mammal; and

c. gestating the embryo in step b., thereby causing the embryo to develop into the transgenic mammal.

11. (Amended) A method of producing a mammalian nuclear transfer embryo, comprising combining a [genome] nucleus from [an] a somatic activated donor cell with an activated,

enucleated oocyte in telophase II and of the same species as the donor cell, to thereby form a nuclear transfer embryo.

15. (Amended) The method of Claim 11, wherein the oocyte is in [Metaphase] metaphase II prior to activation to telophase II.

16. (Amended) The method of Claim 11, wherein the somatic donor cell is activated by reducing nutrients in the serum of the donor cell, and then exposing the donor cell to serum having an increased amount of nutrients.

17. (Amended) The method of Claim 16, wherein the somatic activated donor cell is in a stage of a mitotic cell cycle selected from the group consisting of: G_1 phase, S phase, and G_2/M phase.

19. (Amended) The method of Claim 11, wherein combining a [genomic] nucleus from [an] a somatic activated donor cell with an activated, enucleated oocyte further includes fusing the activated donor cell with the activated oocyte.

20. (Amended) The method of Claim 11, wherein combining a [genomic] nucleus from [an] a somatic activated donor cell with an activated, enucleated oocyte further includes microinjecting the nucleus of the somatic activated donor cell into the activated, enucleated oocyte.

21. (Amended) A method of producing a protein of interest in a mammal [an animal], comprising the steps of:

a. combining a [genome] nucleus from [an] a somatic activated donor cell with an activated, enucleated oocyte in telophase II and of the same species as the donor cell, to thereby form a nuclear transfer embryo, wherein the [genomic] nucleus from the somatic activated donor cell encodes the protein of interest;

b. impregnating [an animal] a mammal of the same species as the nuclear transfer embryo with the nuclear transfer embryo [in] under conditions suitable for gestation of a cloned mammal [animal]; [and]

c. gestating the embryo in step b., thereby causing the embryo to develop into the cloned mammal; and

[c]d. purifying the protein of interest from the cloned mammal [animal].

22. (Amended) The method of Claim 21, wherein purification of the protein of interest is expressed in tissue, cells or bodily secretion of the cloned mammal [animal].

25. (Amended) A method of producing a heterologous protein in a transgenic mammal [animal] comprising the steps of:

a. combining a genetically engineered [genome] nucleus from [an] a somatic activated donor cell with an activated, enucleated oocyte in telophase II and of the same species as the donor cell, to thereby form a nuclear transfer embryo, wherein the [genome] nucleus from the somatic activated donor cell encodes the heterologous protein;

b. impregnating [an animal] a mammal of the same species as the nuclear transfer embryo with the nuclear transfer embryo [in] under conditions suitable for gestation of the nuclear transfer embryo into a cloned mammal [animal]; [and]

c. gestating the embryo in step b., thereby causing the embryo to develop into a cloned mammal that expresses the heterologous protein; and

[c]d. recovering the expressed heterologous protein from the cloned mammal [animal].

28. (Amended) The method of Claim 25, wherein the genetically engineered [genome] nucleus includes an operatively linked promoter.

31. (Amended) A method of enucleating an oocyte having a meiotic spindle apparatus, comprising exposing the oocyte [with] to at least one compound that destabilizes the meiotic spindle apparatus.

39. (New) A method of cloning a mammalian fetus, comprising the steps of:

a. combining a nucleus from a somatic activated donor cell with an activated, enucleated oocyte in telophase II and of the same species as the donor cell, to thereby form a nuclear transfer embryo;

b. impregnating a mammal of the same species as the nuclear transfer embryo with the nuclear transfer embryo under conditions suitable for gestation of the cloned fetus; and

c. gestating the embryo in step b., thereby causing the embryo to develop into the cloned fetus.

40. (New) The method of Claim 39, wherein the activated donor cell is in a stage of a mitotic cell cycle selected from the group consisting of: G_1 phase, S phase, and G_2/M phase.

41. (New) The method of Claim 39, wherein the somatic cell is an adult somatic cell or an embryonic somatic cell.

42. (New) The method of Claim 39, wherein the somatic cell is a fibroblast cell or an epithelial cell.

43. (New) The method of Claim 39, wherein the oocyte is enucleated chemically, by X-ray irradiation, by laser irradiation or by physical removal.

44. (New) A method of cloning a non-human mammal, comprising the steps of:

a. combining a nucleus from a somatic activated donor cell with an activated, enucleated oocyte in telophase II and of the same species as the donor cell, to thereby form a nuclear transfer embryo;

b. impregnating a non-human mammal of the same species as the nuclear transfer embryo with the nuclear transfer embryo under conditions suitable for gestation of the non-human cloned mammal; and

c. gestating the embryo in step b., thereby causing the embryo to develop into the non-human cloned mammal.

45. (New) A method of producing a transgenic non-human mammal, comprising the steps of:

a. combining a genetically engineered nucleus from a somatic activated donor cell with an activated, enucleated oocyte in telophase II and of the same species as the donor cell, to thereby form a transgenic nuclear transfer embryo;

b. impregnating a non-human mammal of the same species as the nuclear transfer embryo with the transgenic nuclear transfer embryo under conditions suitable for gestation of the transgenic non-human mammal; and

c. gestating the embryo in step b., thereby causing the embryo to develop into the transgenic non-human mammal.

46. (New) A method of producing a non-human nuclear transfer embryo, comprising combining a nucleus from a somatic activated donor cell with an activated, enucleated oocyte in telophase II and of the same species as the donor cell, to thereby form a non-human nuclear transfer embryo.

47. (New) A method of cloning a non-human mammalian fetus, comprising the steps of:

a. combining a nucleus from a somatic activated donor cell with an activated, enucleated oocyte in telophase II and of the same species as the donor cell, to thereby form a nuclear transfer embryo;

b. impregnating a non-human mammal of the same species as the nuclear transfer embryo with the nuclear transfer embryo under conditions suitable for gestation of the cloned fetus; and

c. gestating the embryo in step b., thereby causing the embryo to develop into the cloned non-human mammalian fetus.

48. (New) A method of cloning a mammal, comprising the steps of:

a. combining a nucleus from a somatic activated donor cell with an activated, enucleated oocyte derived from an oocyte having a first polar body and an extruding second polar body, and of the same species as the donor cell, to thereby form a nuclear transfer embryo;

b. impregnating a mammal of the same species as the nuclear transfer embryo with the nuclear transfer embryo under conditions suitable for gestation of the cloned mammal; and

c. gestating the embryo in step b., thereby causing the embryo to develop into the cloned mammal.

49. (New) A method of producing a transgenic mammal, comprising the steps of:

a. combining a genetically engineered nucleus from a somatic activated donor cell with an activated, enucleated oocyte derived from an oocyte having a first polar body and an extruding second polar body, and of the same species as the donor cell, to thereby form a transgenic nuclear transfer embryo;

b. impregnating a mammal of the same species as the nuclear transfer embryo with the transgenic nuclear transfer embryo under conditions suitable for gestation of the transgenic mammal; and

c. gestating the embryo in step b., thereby causing the embryo to develop into the transgenic mammal.

50. (New) A method of producing a nuclear transfer embryo, comprising combining a nucleus from a somatic activated donor cell with an activated, enucleated oocyte derived from an oocyte having a first polar body and an extruding second polar body, and of the same species as the donor cell, to thereby form a nuclear transfer embryo.

51. (New) A method of cloning a mammalian fetus, comprising the steps of:

a. combining a nucleus from a somatic activated donor cell with an activated, enucleated oocyte derived from an oocyte having a first polar body and an extruding second polar body, and of the same species as the donor cell, to thereby form a nuclear transfer embryo;

b. impregnating a mammal of the same species as the nuclear transfer embryo with the nuclear transfer embryo under conditions suitable for gestation of the cloned fetus; and

c. gestating the embryo in step b., thereby causing the embryo to develop into the cloned mammalian fetus.

52. (New) A method of cloning a non-human animal, comprising the steps of:

a. combining a nucleus from a somatic activated donor cell with an activated, enucleated oocyte in telophase II and of the same species as the donor cell, to thereby form a nuclear transfer embryo;

b. impregnating a non-human mammal of the same species as the nuclear transfer embryo with the nuclear transfer embryo under conditions suitable for gestation of the non-human cloned mammal; and

c. gestating the embryo in step b., thereby causing the embryo to develop into the non-human cloned mammal.

53. (New) A method of producing a transgenic non-human animal, comprising the steps of:

a. combining a genetically engineered nucleus from a somatic activated donor cell with an activated, enucleated oocyte in telophase II and of the same species as the donor cell, to thereby form a transgenic nuclear transfer embryo;

b. impregnating a non-human mammal of the same species as the nuclear transfer embryo with the transgenic nuclear transfer embryo under conditions suitable for gestation of the transgenic non-human mammal; and

c. gestating the embryo in step b., thereby causing the embryo to develop into the transgenic non-human mammal.

54. (New) A method of producing a non-human nuclear transfer embryo, comprising combining a nucleus from a somatic activated donor cell with an activated, enucleated oocyte in telophase II and of the same species as the donor cell, to thereby form a non-human nuclear transfer embryo.

55. (New) A method of cloning a non-human fetus, comprising the steps of:

a. combining a nucleus from a somatic activated donor cell with an activated, enucleated oocyte in telophase II and of the same species as the donor cell, to thereby form a nuclear transfer embryo;

b. impregnating a non-human mammal of the same species as the nuclear transfer embryo with the nuclear transfer embryo under conditions suitable for gestation of the cloned fetus; and

c. gestating the embryo in step b., thereby causing the embryo to develop into the cloned non-human mammalian fetus.

56. (New) A method of cloning an animal, comprising the steps of:

a. combining a nucleus from a somatic activated donor cell with an activated, enucleated oocyte derived from an oocyte having a first polar body and an extruding second polar body, and of the same species as the donor cell, to thereby form a nuclear transfer embryo;

b. impregnating a mammal of the same species as the nuclear transfer embryo with the nuclear transfer embryo under conditions suitable for gestation of the cloned mammal; and

c. gestating the embryo in step b., thereby causing the embryo to develop into the cloned mammal.

57. (New) A method of producing a transgenic animal, comprising the steps of:

a. combining a genetically engineered nucleus from a somatic activated donor cell with an activated, enucleated oocyte derived from an oocyte having a first polar body and

an extruding second polar body, and of the same species as the donor cell, to thereby form a transgenic nuclear transfer embryo;

b. impregnating a mammal of the same species as the nuclear transfer embryo with the transgenic nuclear transfer embryo under conditions suitable for gestation of the transgenic mammal; and

c. gestating the embryo in step b., thereby causing the embryo to develop into the transgenic mammal.

58. (New) A method of producing a non-human nuclear transfer embryo, comprising combining a nucleus from a somatic activated donor cell with an activated, enucleated oocyte derived from an oocyte having a first polar body and an extruding second polar body, and of the same species as the donor cell, to thereby form a nuclear transfer embryo.

59. (New) A method of cloning a fetus, comprising the steps of:

a. combining a nucleus from a somatic activated donor cell with an activated, enucleated oocyte derived from an oocyte having a first polar body and an extruding second polar body, and of the same species as the donor cell, to thereby form a nuclear transfer embryo;

b. impregnating a mammal of the same species as the nuclear transfer embryo with the nuclear transfer embryo under conditions suitable for gestation of the cloned fetus; and

c. gestating the embryo in step b., thereby causing the embryo to develop into the cloned mammalian fetus.